STANDARD PROCEDURES FOR BRUCELLOSIS TESTING AT LIVESTOCK MARKETS
SPECIFICALLY APPROVED UNDER TITLE 9, PART 78, CFR,
GOVERNING INTERSTATE MOVEMENT OF CATTLE WITH REGARD TO BRUCELLOSIS

Brucellosis testing conducted at approved livestock markets shall be carried out in such a manner as to assure maximum accuracy of results. In order that this may be accomplished, it is essential that recommended practices be followed. It is likewise important that operators be fully qualified in setting up tests and interpreting results.

All laboratories located at approved livestock markets shall be mutually acceptable to the State livestock sanitary officials and Federal veterinarians in charge of ADE activities from the standpoint of space and equipment. Personnel authorized to conduct tests shall have demonstrated their ability in this regard to the satisfaction of both State and Federal officials. It is advisable that necessary training be carried out at the cooperative brucellosis testing laboratory within the State.

I. Laboratory Equipment

a. Sufficient bleeding needles to permit use of a separate clean, dry needle for collecting each sample.

b. Standard bleeding tubes, 3 to 4 inches in length, with rubber stoppers.

c. Sufficient pipettes to permit use of an individual pipette for each sample tested.

d. Centrifuge capable of handling the required volume of daily tests. A speed of 1500 rpm is recommended.

e. Standard brucellosis testing box, or equivalent, with plates having $1\frac{1}{4}$ inch square markings.

f. Multiple stirring device for mixing serum-antigen mixtures.

g. Approved antigen dropping device delivering uniform 0.03 ml drops. Antigen in 30 cc bottles and manual bulb-type droppers to fit such bottles are available through the Veterinarian in Charge, ADE Division, in your State.

II. Testing Procedure

a. Collect blood samples as aseptically as possible in clean vials. Note: Sufficient blood should be obtained from each animal so that at least 5 ml of whole blood or 2 ml of clear serum can be forwarded to the central cooperative laboratory for check testing of all animals bled.

b. Test only clean sera samples. If necessary, break down clots and centrifuge to obtain suitable specimens.

c. Screen all samples at a 1:50 dilution or less by the use of a standard pipette or micro-pipette adequately washed between samples. (Micro-pipettes may be obtained from most major laboratory supply companies.) The screening dilutions (not less than 0.04 ml serum plus 0.03 ml antigen) are mixed, rotated, and incubated as described below.

d. All screen dilutions exhibiting any reactions shall be rerun, using a separate clean pipette on each sample, in the three recognized dilutions, namely:

1:50 - 0.04 ml serum / 0.03 ml antigen
1:100 - 0.02 ml serum / 0.03 ml antigen
1:200 - 0.01 ml serum / 0.03 ml antigen

e. No more than 12 samples should be run in a single group.

f. Multiple mixing of samples shall start at the 1:200 dilution and proceed through the 1:100 and finally the 1:50 concentrations.

g. The mixing device should be thoroughly rinsed and dried between samples.

h. Serum-antigen mixtures must be rotated gently at the following periods:

1. At beginning of incubation period.

2. Four minutes later.

3. At end of incubation period (8 minutes) and just prior to reading tests.

4. All tests shall be read against a uniform reflected light similar to that provided in the standard brucellosis test box.

i. Interpretations

Table 1. - For unvaccinated cattle (unchanged)

Dilutions			Diagnosis
1:50	1:100	1:200	
-*	-	-	Negative
I	-	-	Suspect
/	-	-	Suspect
/	I	-	Suspect
/	/**	-	Reactor

Table 2. - For calf-vaccinated cattle (new interpretation)

Dilutions			Diagnosis
1:50	1:100	1:200	
/*	-	-	Negative
/	I	-	Suspect
/	/	-	Suspect
/	/	I	Suspect
/	/	/**	Reactor

Key to both tables:
- = Negative
/ = Complete agglutination
I = Incomplete agglutination
* = or less
** = or higher

j. Permanent records shall be maintained on all tests, showing identifications, origins and destinations of animals involved. These records are in addition to those reported on official charts and certificates.

k. Arrangements will be made with the central cooperative testing laboratory for submission of all samples for duplicate testing of all animals bled.

III. Precautions to Be Observed

a. Hemolyzed samples cannot be accurately tested. Freezing, high temperatures and contamination are common causes of hemolysis.

b. Refrigerate antigen when not in use.

c. Allow antigen to reach room temperature before use.

d. Check antigen droppers to assure accurate delivery of 0.03 ml per drop. (100 drops should equal 3 ml.)

e. Tests should be conducted at temperatures ranging between 70 and 78 degrees F. (Extremes of either heat or cold may result in incorrect interpretations.)

f. Only thoroughly clean, dry needles should be used in collecting blood samples.

g. Each pipette should be suitably sterilized and dried before use. Note: Sterilization can be accomplished either chemically or by heat.

h. It is extremely important that all blood samples be accurately identified at the time they are collected and that the same identification be carried through on samples that are submitted for check testing.

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